Sensitizing processing-resistant foodborne spoilage and pathogenic bacteria to ultra-high pressure by food colorants

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ABSTRACT

Treating foods with ultra-high pressure (UHP) causes significant inactivation of microbial load, however a small number of survivors are often observed after extreme pressure treatments (a tailing phenomenon). Consequently, it is important to identify conditions and additives that enhance the efficacy of pressure against foodborne pathogenic and spoilage microorganisms. Based on preliminary work, it was hypothesized that food colorants could be used in combination with UHP to enhance pressure lethality.

The seven U.S. certified food colorants (FD&C Blue No. 1, Blue No. 2, Green No. 3, Red No. 3, Red No. 40, Yellow No. 5, and Yellow No. 6) were tested for synergy with UHP against *Lactobacillus plantarum* in citrate-phosphate buffer (pH 7.0).

FD&C Red No. 3 was the only food colorant exhibited antimicrobial properties as well as enhancing UHP lethality. Further studies were performed using FD&C Red No. 3 against processing-resistant *Listeria monocytogenes* and *Escherichia coli* O157:H7 strains. Synergy of inactivation occurred with FD&C Red No. 3 concentrations as few as 3 ppm when the pathogens were treated with 400 MPa for 3 minutes. FD&C Red No. 3 (10 ppm) and UHP (400 MPa for 3 minutes) inactivated a large population (>7 log cfu/ml) of the processing-resistant *L. monocytogenes* OSY-328; UHP treatment alone inactivated ~0.3 log cfu/ml, whereas the treatment alone inactivated 1.8 log cfu/ml.

Gram-positive strains were sensitive to FD&C Red No. 3 alone, while *E. coli* O157:H7 was only affected when treated with the colorant in combination with UHP.

INTRODUCTION

Considerable variability among microorganisms in barotolerance has been demonstrated at the genus, species and strain levels (2). Barotolerant strains of *Listeria monocytogenes* (8), *Lactobacillus plantarum*, *Lactobacillus fermentum* (10), and *Escherichia coli* O157:H7 (3) pose potential risk in food processed by pressure. Consequently, it is important to identify conditions and additives that enhance the efficacy of pressure against foodborne pathogenic and spoilage microorganisms. Recently, investigators screened a large number of food additives for synergy with high pressure against foodborne pathogens. Synergistic inactivation with high pressure has been found with nisin, lysozyme, pediocin, lactoferrin, and lactoferricin against both Gram-positive and Gram-negative bacteria (4,5). Chung et al. (1) and Yurma et al. (9) found that phenolic antioxidants, particularly TBHQ, sensitize *Listeria monocytogenes* to pressure.

Food colorants, which are traditionally used to impart a desired color to a food product, are tested in this study. Previous studies have shown potential antimicrobial properties for coloring agents, mostly focusing on xanthene-derivatives (including FD&C Red No. 3) (6,7,11).

Previously in the laboratory, red food coloring was used as a tool to verify homogenization of an inoculum in ranch-style salad dressing and subsequent high pressure treatment. Inclusion of the food coloring resulted in exceptionally poor recovery of cells from the sample with and without ultra-high pressure treatment. Based on this observation, further investigation into the antimicrobial activity of food dyes and potential synergy with ultra-high pressure processing was investigated.

OBJECTIVES

- Determine the effect of combination treatments of certified food colorants with ultra-high pressure against *Lactobacillus plantarum* strains.
- Investigate dose-response of FD&C Red No. 3 with and without pressure treatment against processing-resistant spoilage and pathogenic bacteria.

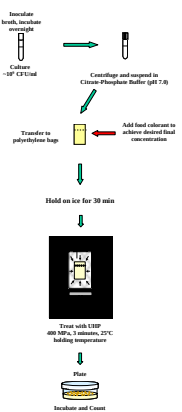
METHODS

Bacterial Strains

Table 1. Bacterial species and strains that vary in resistance to UHP treatment to be analyzed for inactivation by food coloring and UHP combination treatments.

Species	Strain	Relative UHP-resistance
Lactobacillus plantarum	MDOS-32	UHP-resistant
Lactobacillus plantarum	OSY-104	UHP-sensitive
Listeria monocytogenes	ScottA	UHP-sensitive
Listeria monocytogenes	OSY-328	UHP-resistant
Escherichia coli O157:H7	EDL 933	Moderate UHP-resistance
Escherichia coli O157:H7	EDL 933 - A	UHP-resistant mutant
Escherichia coli O157:H7	EDL 933 - C	UHP-resistant mutant

Culture preparation and pressure treatment procedure



RESULTS

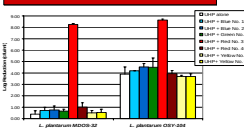


Fig. 1. Log inactivation of *Lactobacillus plantarum* strains in response to ultra-high pressure (400 MPa, 3 min) in combination with 300 ppm of each of the seven certified food colorants. Initial cell counts were ~10^9 cfu/ml; n = 3; error bars represent standard error.

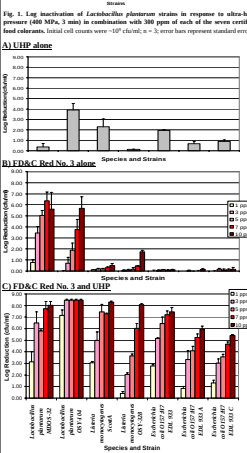


Figure 2. Log reduction of *Lactobacillus plantarum*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 strains in response to ultra-high pressure at 400 MPa for 3 min (A), varying doses of FD&C Red No. 3 (B), and combination treatments of ultra-high pressure and FD&C Red No. 3 (C). Initial cell counts were ~10^9 cfu/ml; n = 3; error bars represent standard error.

DISCUSSION

- FD&C Red No. 3 was the only certified food colorant (at 300 ppm) to enhance inactivation of *Lactobacillus plantarum* by ultra-high pressure (Figure 1).
- FD&C Red No. 3 (at 300 ppm) was also effective at reducing populations of *Lactobacillus plantarum* without pressure treatment (data not shown).
- Species and strains were compared for relative barotolerance (Figure 2A). Inactivation of these microorganisms were comparable to previous studies. Overall, *Lactobacillus plantarum* OSY-104 was the most sensitive strain to ultra-high pressure and *Listeria monocytogenes* OSY-328 was the most resistant under these conditions.
- Species and strains showed varying levels of inactivation when treated with FD&C Red No. 3 alone (up to 10 ppm) (Figure 2B).
  - *Lactobacillus plantarum* strains were the most sensitive to colorant exposure, with inactivation being dose-dependent. *Lactobacillus plantarum* OSY-104 tended to be more resistant to the colorant than *L. plantarum* MDOS-32.
  - *Listeria monocytogenes* strains were also sensitive to colorant exposure, but more resistant that *L. plantarum* strains. *Listeria monocytogenes* ScotA appears to be slightly more resistant to FD&C Red No. 3 when compared to *L. monocytogenes* OSY-328.
  - *Escherichia coli* O157:H7 strains were unaffected by FD&C Red No. 3 alone. Concentrations of FD&C Red No. 3 were increased to 300 ppm producing no inactivation (data not shown).
- All species and strains displayed synergistic inactivation by the combination of FD&C Red No. 3 and ultra-high pressure treatment. The inactivation by combination treatment was FD&C Red No. 3 dose-dependent (Figure 2C).
  - Synergy was most apparent with *E. coli* O157:H7 strains due to their inherent resistance to FD&C Red No. 3 treatment. This finding likely indicates the effect of ultra-high pressure on destabilization of the outer membrane of Gram-negative bacteria. Destabilization may allow access of the colorant to a compartment of the cells where toxic effect occurs.

Conclusion:

FD&C Red No. 3 in combination with ultra-high pressure processing is effective at reducing populations of important processing-resistant food spoilage and pathogenic bacteria. This treatment combination could be used in food products to enhance safety and/or delay spoilage.

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